

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 216 133 B1

(12)

EUROPEAN PATENT SPECIFICATION

(43) Date of publication of patent specification: 28.07.93 (51) Int. Cl.⁵: **A61K 35/72, A61K 31/70**

(21) Application number: **86111411.4**

(22) Date of filing: **18.08.86**

(54) **Nutritional agent for the cerebrospinal nervous system.**

(30) Priority: **16.08.85 JP 180146/85**

(43) Date of publication of application:
01.04.87 Bulletin 87/14

(45) Publication of the grant of the patent:
28.07.93 Bulletin 93/30

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
EP-A- 0 149 775

(73) Proprietor: **NISSAN CHEMICAL INDUSTRIES LTD.**

**3-7-1, Kanda Nishiki-cho
Chiyoda-ku Tokyo(JP)**

(72) Inventor: **Morishige, Fukumi**
**3-30-2, Kamikitazawa Setagaya-ku
Tokyo(JP)**

(74) Representative: **Patentanwälte Schaad, Balass
& Partner**
**Dufourstrasse 101 Postfach
CH-8034 Zürich (CH)**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Description**BACKGROUND OF THE INVENTION**

1. Field of the Invention

This invention relates to a nutritional agent which promotes recovery from conditions of cerebrospinal degenerative diseases, such as epilepsy, an attack of convulsion, brain degenerative disease, cranial nerve disease, cerebral basal ganglion disease, cerebellar degenerative disease, spinal degenerative disease and muscular disease.

2. Description of the Prior Art

In recent years, there has been a striking advance in the-diagnostic and therapeutic techniques in the field of cranial nerve surgery for example CT (computer tomography) scanning, ultrasonication, cerebral angiography and micro-surgery, but there have been few satisfactory therapeutic agents for dementia, hypomnesia, epilepsy, an attack of convulsion, which are due to a decrease in the function of nervous cells of the brain.

Ribonucleic acid has been used in the treatment of mental retardation and to improve memory MARTINDALE 28Ed. (1982) p. 1751. Dried yeast was applied for the prevention of vitamin-B deficiency (loc.cit. p. 1641). In the EP-A-149 775 a composition of nucleic acid components for use with patients with impaired metabolism owing to liver diseases, gastrointestinal diseases is described.

SUMMARY OF THE INVENTION

In view of the above state of the art, the present invention provides a nutritional agent useful for the promotion of recovery of cerebrospinal diseases, which comprises ribonucleic acid (to be referred to as RNA), preferably derived from yeast, together with pharmaceutically acceptable salt of calcium or magnesium.

Examples of the cerebrospinal degenerative diseases are epilepsy, an attack of convulsion, brain degenerative disease, cranial nerve disease, cerebral basal ganglion disease, cerebellar degenerative disease, and muscular diseases. The present inventors searched and studied a nutritional agent which improves these conditions. Unexpectedly, the present inventors have found that by orally administering a nutritional agent comprising RNA to the patient of such a condition, the condition is improved, and accomplished the present invention.

Specific examples of cerebrospinal degenerative disease on which the nutritional agent of this invention is effective include epilepsy; an attack of convulsion; and brain degenerative diseases, for example degenerative diffuse sclerosis such as Pelizaeus-Merzbacher disease, Sholtz disease, Krabbe disease, other congenital hereditary diseases amaurotic familiar, Rourence-Moon-Bordet-Bied syndrome, Hurler syndrome, cerebral disease in the old age and in the early old age (such as senile dementia, Alzheimer disease, and Pick disease), and cranial nerve diseases such as progressive bulbar palsy, progressive paralysis of ocular muscles, hereditary nystagmus, Adie syndrome, syringobulbia, Leber disease and cerebral basal ganglion disease such as Parkinson's disease, Chorea, dystonia syndrome, athetosis syndrome, myoclonia syndrome, artuun Parkinsonism, degenerative diseases of the cerebellum such as hereditary, spinal and cerebellar ataxia, hereditary ataxia with myoatrophy, muscular diseases such as progressive muscular dystrophy, myoasthenia, and periodic paralysis.

RNA as active ingredients of the nutritional agent of this invention may be of natural origin or synthetic materials.

RNA, of natural origin mean those obtained from microbial cells, preferably RNA, extracted and fractionated from yeasts such as brewery yeast, baker's yeast, Torula yeast and milk yeast, especially preferably RNA, extracted from brewery yeast.

The method of obtaining RNA, of natural origin is exemplified by the following method starting from brewery yeast.

The residue resulting from removal of water-soluble low-molecular-weight fractions from a slurry-like (solids content about 10 % by weight) or dry yeast recovered from the beer producing process is used as a starting material. An aqueous solution of sodium chloride is added to the residue, and RNA is extracted under heat. Concentrated hydrochloric acid is added to the extract to form a precipitate. After neutralization, extraction is again effected by adding water. Then, an organic solvent which is difficult of dissolving RNA,

such as ethanol, is added to precipitate RNA. The supernatant is removed by centrifugal separation and a precipitated fraction composed mainly of RNA is obtained. It is then dried by a suitable conventional method.

The approximate composition of brewery yeast RNA after drying is as follows:

5

<u>Ingredient</u>	<u>Content (dry base)</u>
RNA (*1)	65 - 95 % by weight
CAS (*2)	less than 5
DNA	less than 1
Proteins	less than 22
Sugar (hexose)	less than 18
Sodium chloride	less than 0.5
(*1): Molecular weight	1.3×10^4
(*2): Low-molecular-weight ribonucleic acid,	
ribonucleotide and ribonucleoside which are	
soluble in 5 % cold perchloric acid.	

20

25

30

The oral toxicity to mice of ribonucleic acid extracted from yeast can be said to be very low since it exerts no action even when administered in a dose of 5250 mg/kg.

35

The nutritional agent in accordance with this invention comprises RNA, themselves, or a mixture of RNA, with at least one suitable nutritionally or pharmacologically acceptable adjuvant such as a binder (syrup, gum arabic, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone, a vehicle (lactose, sugar, corn starch, calcium phosphate, sorbitol, glycine, a lubricant (magnesium stearate, talc, polyethylene glycol, silica, a disintegrant (potato starch, and a flavor or a sweetener (sugar, aspartame, saccharin, in the form of a powder, granule, tablets or capsules.

40

The nutritional agent of this invention is desirably administered orally.

The dosage for administration to adults is 0.1 to 2.7 g, preferably 0.5 to 1.5g as RNA, once, or two or three times in divided dosages, daily. The dosage, however, may be increased or decreased depending upon the age, body weight, condition, etc., of the patient.

45

Addition of pharmaceutically acceptable calcium and magnesium salts to RNA, tends to enhance the nutritional or pharmacological activity of RNA. Examples of these pharmaceutically acceptable calcium and magnesium salts are those in which the acid portion is almost inert pharmacologically and the metal ion portion alone can be expected to have activity, such as calcium lactate and magnesium sulfate. The weight ratio of these salts to the total weight RNA, depends upon the weight of the calcium or magnesium portion, and is, for example, 1 to 5 for calcium lactate, and 1 to 5 for magnesium sulfate.

50

When the nutritional agent of this invention is administered in admixture with ascorbic acid, sodium ascorbate, or calcium ascorbate, the uric acid value of the blood tends to decrease from that in the absence of such an additional compound. Thus, with patients of such a constitution that the administration of the nutritional agent of this invention tends to increase the uric acid value, a symptom of gout which may be induced by the rise of the blood uric acid value can be avoided.

55

The ratio of ascorbic acid, sodium ascorbate or calcium ascorbate to the total weight of RNA, is from 0.5 to 10, preferably from 1 to 5.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will be described in greater detail on the basis of the following examples. All percentages in these examples are by weight.

Production Example 1

(1) The slurry-like yeast recovered from the beer production process was passed through a sieve having a size of 80 to 200 mesh to remove solids. The residue was washed with an aqueous solution of sodium carbonate and then water to obtain a raw yeast deprived of bitter taste.

(2) Sodium chloride and water were added so as to provide a yeast concentration of 10 % and a sodium chloride concentration of 10 %. The mixture was heated and boiled for 2 to 5 hours. Instead of this boiling, autoclaving for 1 hour may be carried out.

(3) Ribonucleic acid was extracted under boiling. Cooling and solid-liquid separation by a centrifugal separator gave an extract.

The solid containing ribonucleic acid was washed with 10 % sodium chloride solution, and the wash liquor was recovered and combined with the extract.

(4) Conc. hydrochloric acid was added to this extract to adjust to pH 2, and a fraction precipitated under acidity was obtained. It was separated and recovered, and sodium hydroxide was added to form a precipitate again. Since this precipitate was rapidly formed, the supernatant was removed, and the precipitate was recovered by a centrifugal separator. The resulting extract was dehydrated with 98 % ethanol and washed, and dried by a suitable method.

Production Example 2

Water (450 liters) was added to 90 kg of dry brewery yeast, and the mixture was stirred for a predetermined period of time to solubilize impurities and wash the yeast.

The mixture was then centrifuged to recover washed yeast. Water (800 liters) and 90 kg of sodium chloride were added, and the mixture was heated. The mixture was boiled for 2 hours and subjected to a centrifugal separator. The residue containing ribonucleic acid was washed with 10 % aqueous sodium chloride solution, and the wash liquor was recovered. The wash liquor was combined with the extract, and by performing the same treatment as (4) of Production Example 1, brewery yeast was obtained.

Production Example 3

A slurry-like brewery yeast was deprived of a bitter taste and washed by a customary method, and dried on a drum dryer at 120 to 140 °C. Hot water at a temperature of 95 °C or higher was added to the dry brewery yeast in an amount ten times the latter. The mixture was maintained for 5 minutes, and subjected to solid-liquid separation to obtain a solid. Sodium chloride and water were added to the solid, and the concentration of the solid brewery yeast and the concentration of sodium chloride were each adjusted to 10 %. The solution was fed to a homogenizer so that the discharge pressure became at least 600 kg/cm² in order to crush the cellular wall. The treated liquid containing the crushed yeast was heated and boiled for 2 to 5 hours, and thereafter, the same treatments as in (3), and (4) of Production Example 1 to obtain crude ribonucleic acid from brewery yeast.

The results of analyzing the compositions of crude RNAs obtained by these Production Examples are shown in the following table.

Production Example	1	2	3
Yield (%) of crude ribonucleic acid	1.51	2.57	3.09
Components (%) of crude ribonucleic acid			
CAS fraction*	3.0	6.0	5.4
ribonucleic acid fraction*	87.4	77.1	85.6
DNA fraction*	0	1.2	1.32

* These components were fractions analyzed by the Schmidt Townhouser and Schnider method (STS method) (Food Analysis Method edited by the Committee of Editing Food Analysis Methods, Japanese Society of Food Industry, p. 563, 1982). CAS is an acid-soluble low-molecular-weight ribonucleic acid of nucleotide and nucleoside.

Test Example 1

Acute Toxicity Test:

Brewery yeast RNA (RNA content 70 %) obtained in accordance with the method of Production Example 1 was tested for acute toxicity by forced oral administration to mice (ICR strain, 40 males and 40 females). Before administration, the animals were caused to fast for 18 hours. A suspension of 7500 mg (5250 mg calculated as RNA) of the brewery yeast in 50 ml of water was administered per kilogram of the animals, and then the animals were observed for 14 days. But no abnormality was noted.

In the following Clinical Examples, RNA represents the total amount of RNA^(*) and CAS^(*) described in the table on page 4. The brewery yeast RNA was produced from the material produced in accordance with Production Example 1.

Clinical Example 1

Example of promotion of recovery of a patient with epilepsy:

The patient was a 7-year old girl with the following history. About 1 year ago, she got epilepsy. Nearly extreme doses of an antiepileptic were administered, but epileptic convulsion did not cease. The doctor told the parents that prognosis was bad. She was unable to take foods, and nutrition was maintained through a stomach tube. Her consciousness was not clear but always hypalgescic. She incessantly repeated epileptic convulsion. Brain computer tomography showed marked enlargement of the ventricle and compression and atrophy of the brain.

An agent containing the brewery yeast RNA (RNA content 70 %) was administered to the patient in a dose of 3 g/day in three divided dosages over the course of 6 months. One week after the starting of

administration of the brewery yeast RNA, epileptic convulsion was not observed. A basal drainage was inserted in order to remedy the enlargement of the ventricle. After hospitalization for about 40 days, the patient was allowed to leave the hospital. Thereafter, an agent containing brewery yeast RNA (RNA content 70 %) was administered in the same dosage as above. The patient became able to take foods by her own power, and began to laugh. About 6 months after the starting of administration of the RNA-containing agent, epileptic convulsion quite disappeared.

Clinical Example 2

Example of treating Parkinson's disease:

The patient was an 81-year old person with Parkinson's disease and had the following history. A reduction in spontaneity, urine incontinence, a light degree of dementia and derangements in emotion or volition were noted, and the patient used a wheeled chair. The patient was treated with drugs including E-C-dopal®, ARTANE® and Uvela N® and caused to exercise rehabilitation, but not significant effect was noted.

An agent containing brewery yeast RNA (RNA content 70 %) was administered to the patient in a dose of 3 g per day in three divided dosages over the course of 3 months. Then, the patient could walk by himself, and an increase in feeling, action and spontaneity was observed. For example, the patient could go to a nearby dentist or go shopping in nearby stores.

(Nuclear C_{RS} in the following clinical example was produced from the material produced in accordance with Production Example 1. The definition of RNA was the same as in the above Clinical Examples.)

Clinical Example 3

Example of reduction in the attack of convulsion:

The patient was a two-year old infant who was born by uneventful delivery, but was infected with encephalitis by viral infection (herpes I type).

The patient was infected with encephalitis by viral infection (herpes I type) seven months after birth. Two to three months after infection, an attack of convulsion occurred in the patient. An anticonvulsant was administered, but found to be ineffective. hemiplegia occurred as a complication, and an attack of epilepsy lasted for a whole day. Nutrition was administered through a stomach tube.

The results of diagnosis about 2 years after birth were as follows:

"Incessant convulsion was observed, and the patient was senseless. hemiplegia was noted, and the Babinski reflex was weakly positive. Brain computer tomography showed that the right right temporal lobe was close to the Sylvian's fissure, and a low concentration was observed, and that no enlargement of the side ventricle was noted"

About three spoonfuls (about 5.4 g; containing 1.4 g of RNA derived from brewery yeast and 3.0 g of vitamin C) of Nuclear C_{RS}, 1.0 g of calcium lactate and 1.0 g of magnesium sulfate were administered to the patient per day, but convulsion did not decrease.

Beginning approximately on the 50th day after the starting of administering the RNA-containing agent, about 6 spoonfuls (about 10.8 g containing 2.7 g of RNA derived from brewery yeast and 6.0 g of vitamin C) of Nuclear C_{RS}, 2.0 g of calcium lactate, and 2.0 g of magnesium sulfate per day were administered. By diagnosis two weeks later, the convulsion was markedly reduced. Thirty convulsions per day now decreased to 10 to 14 convulsions per day, and his facial expression improved, and became good in temper. About 4 months and 20 days after the starting of administering the RNA-containing agent, the attack of convulsion further showed a decreasing tendency.

Clinical Example 4

Example of decreasing attacks of convulsion:

The patient was a 21-year old male infected with viral meningitis during infancy:

The patient was first treated with a general anticonvulsant, but an attack of convulsion continued. He was grown in a certain institution.

The patient was hospitalized, and six spoonfuls (about 10.8 g containing 2.7 g of RNA derived from brewery yeast and 6.0 g of vitamin C) of Nuclear C_{RS}, 180 mg of VB₆, 3 g of VB₃ (bulk), 1 to 2.0 g of calcium lactate and 2.0 g of magnesium sulfate were administered daily.

About one month after the starting of administering the RNA-containing agent, the attack was alleviated. Since the anticonvulsant was of the barbiturate type, the patient tended to be drowsy. Gradually, the administration of the drugs previously taken was stopped, and 6 spoonfuls (about 10.8 g containing 2.7 g of RNA derived from brewery yeast and 6.0 g of vitamin C) of Nuclear C_{RS}, 180 mg of VB₆, 3 g of VB₃ (bulk),
 5 2.0 g of calcium lactate and 2.0 g of magnesium sulfate (not containing any anticonvulsant) were administered daily. Now, a tendency toward particularly frequent convulsion during fever has disappeared.

Clinical Example 5

10 Example of decreasing attacks of convulsion:

At the time of delivery the ligament wrapped around the neck portion of the patient and was born in a synoptic state, but resuscitation was successful.

About 2 months after birth, convulsion occurred and continued to date. An anticonvulsant was
 15 administered by a nearby physician, but was not effective. A stomach tube was inserted. As a result of high doses of a strong sedative, particularly a barbiturate preparation, the patient always shows a drowsy tendency. Nutrition is administered through the stomach tube.

The results of diagnosis about 8 months and 25 days after birth were as follows:

"Muscular hypertonicity of the entire body was noted. Primitive reactions which should disappear at this
 20 age, such as Moro reflex, grasp reflex, and plantar reflex remained to some extent, and no sucking reflex. The Babinski reflex which at this age shows a derangement of the pyramidal tract was observed only at the left lower extremity. The patient cannot sit down alone, nor toss on a bed. His eyesight was deranged."

About one spoonful (about 1.8 g containing 0.5 g of RNA derived from brewery yeast and 1.0 g of vitamin C) of Nuclear C_{RS} was administered orally to this patient three times daily, and at the same time,
 25 1.0 g of calcium lactate and 1.0 g of magnesium sulfate were administered. Anticonvulsants prescribed by a nearby physician were administered excepting a barbiturate preparation. The attack of convulsion gradually decreased markedly, and therefore, the anticonvulsants were further decreased. But convulsion did not increase, while the patient previously was always drowsy, he began to laugh upon been called and stand up about 2 months after the starting of administering the RNA-containing agent. Furthermore, the patient began
 30 to play with toys, and asked for maternal husking.

Now, convulsion exists little before sleep.

Formulation examples are shown below. The definition of RNA was the same as in the above Clinical Examples.

35 Formulation Example 1

One gram of brewery yeast RNA obtained by the method of Production Example was enveloped in one pack.

40 Formulation Example 2

Sodium ascorbate (3.0 g) was mixed with 1.0 g of brewery yeast RNA obtained by the method of Production Example 1, and the mixture was enveloped in one pack.

45 Formulation Example 3

Brewery yeast RNA (1.0 g) obtained by the method of Production Example 1 was mixed with 3.0 g of sodium ascorbate, 1.0 g of calcium lactate and 1.0 g of magnesium sulfate, and the mixture was enveloped into one pack.

50 Formulation Example 4

Brewery yeast RNA (1.0 g) obtained by the method of Production Example 1 was mixed with 1.0 g of calcium lactate and 1.0 g of magnesium sulfate, and the mixture was enveloped into one pack.

55 As is clearly seen from the foregoing Clinical Examples, the nutritional agent of this invention is effective for promoting improvement and recovery of conditions of cerebrospinal degenerative diseases, such as epilepsy, Parkinson's disease and an attack of convulsion.

Claims

1. Use of pharmaceutically acceptable salt of calcium or magnesium together with ribonucleic acid for the manufacture of a nutritional agent which is useful for the alleviation or disappearance of an attack of epilepsy.
2. Use of pharmaceutically acceptable salt of calcium or magnesium together with ribonucleic acid for the manufacture of a nutritional agent which is useful for the alleviation or disappearance of Parkinson's disease.
3. Use of pharmaceutically acceptable salt of calcium or magnesium together with ribonucleic acid for the manufacture of a nutritional agent which is useful for the alleviation or disappearance of an attack of convulsion.
4. Use of pharmaceutically acceptable salt of calcium or magnesium together with ribonucleic acid for the manufacture of a nutritional agent which is useful for recovering or improving the function of the cerebrospinal nerves decreased from the normal function.
5. The use of any one of the claims 1 to 4 wherein the ribonucleic acid is a ribonucleic acid extracted and fractionated from a yeast.
6. The use of claim 5 wherein the yeast is a brewery yeast.
7. The use of claim 1 wherein the attack of epilepsy is an attack symptomatic epilepsy.
8. The use of claim 1 wherein the attack of epilepsy is an attack of genuine epilepsy.
9. The use of claim 3 wherein the attack of convulsion is attributed to a cerebrospinal degenerative disease.

Patentansprüche

1. Verwendung eines pharmazeutisch annehmbaren Salzes von Calcium oder Magnesium zusammen mit Ribonucleinsäure zur Herstellung eines Nahrungsmittels, das geeignet ist, einen Epilepsieanfall zu erleichtern oder zum Verschwinden zu bringen.
2. Verwendung eines pharmazeutisch annehmbaren Salzes von Calcium oder Magnesium zusammen mit Ribonucleinsäure zur Herstellung eines Nahrungsmittels, das geeignet ist, die Parkinson'sche Krankheit zu erleichtern oder zum Verschwinden zu bringen.
3. Verwendung eines pharmazeutisch annehmbaren Salzes von Calcium oder Magnesium zusammen mit Ribonucleinsäure zur Herstellung eines Nahrungsmittels, das geeignet ist, einen Krampfanfall zu erleichtern oder zum Verschwinden zu bringen.
4. Verwendung eines pharmazeutisch annehmbaren Salzes von Calcium oder Magnesium zusammen mit Ribonucleinsäure zur Herstellung eines Nahrungsmittels, das geeignet ist, die normale Funktion der cerebrospinalen Nerven, deren Funktion verschlechtert ist, wieder herzustellen oder zu verbessern.
5. Verwendung nach einem der Ansprüche 1 bis 4, wobei die Ribonucleinsäure eine aus Hefe extrahierte und fraktionierte Ribonucleinsäure ist.
6. Verfahren nach Anspruch 5, wobei die Hefe eine Bierhefe ist.
7. Verwendung nach Anspruch 1, wobei der Epilepsieanfall ein Anfall von symptomatischer Epilepsie ist.
8. Verwendung nach Anspruch 1, wobei der Epilepsieanfall ein Anfall von genuiner Epilepsie ist.

9. Verwendung nach Anspruch 3, wobei der Krampfanfall auf eine cerebrospinale degenerative Erkrankung zurückzuführen ist.

Revendications

5

1. Utilisation d'un sel pharmaceutiquement acceptable de calcium ou de magnésium avec un acide ribonucléique pour la fabrication d'un agent nutritionnel utile pour l'atténuation ou la disparition d'une crise d'épilepsie.
- 10 2. Utilisation d'un sel pharmaceutiquement acceptable de calcium ou de magnésium avec un acide ribonucléique pour la fabrication d'un agent nutritionnel utile pour l'atténuation ou la disparition de la maladie de Parkinson.
- 15 3. Utilisation d'un sel pharmaceutiquement acceptable de calcium ou de magnésium avec un acide ribonucléique pour la fabrication d'un agent nutritionnel utile pour l'atténuation ou la disparition d'une crise de convulsions.
- 20 4. Utilisation d'un sel pharmaceutiquement acceptable de calcium ou de magnésium avec un acide ribonucléique pour la fabrication d'un agent nutritionnel utile pour guérir ou améliorer le fonctionnement des nerfs cérébrospinaux dont le fonctionnement est détérioré par rapport à la normale.
5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle l'acide ribonucléique est un acide ribonucléique extrait et fractionné à partir d'une levure.
- 25 6. Utilisation selon la revendication 5, dans laquelle la levure est une levure de bière.
7. Utilisation selon la revendication 1, dans laquelle la crise d'épilepsie est une crise d'épilepsie symptomatique.
- 30 8. Utilisation selon la revendication 1, dans laquelle la crise d'épilepsie est une crise d'épilepsie congénitale.
9. Utilisation selon la revendication 3, dans laquelle la crise de convulsions est attribuée à une maladie dégénérative cérébrospinale.

35

40

45

50

55